



SEQUENCE LISTING

<110> HARTMANN, ARNO
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SOBEL, CORNELIUS
LO, KIN-MING
WAY, JEFFREY C.
GILLIES, STEPHEN

<120> ERYTHROPOIETIN FORMS WITH IMPROVED PROPERTIES

<130> MERCK-2056

<140> 09/708,506

<141> 2000-11-09

<150> 60/164,855

<151> 1999-11-12

<160> 29

<170> PatentIn Ver. 2.1

<210> 1

<211> 514

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (8)..(505)

<223> Human EPO, DNA sequence modified but no change in
protein sequence

<400> 1

cccgggt	gcc	cca	cca	cgc	ctc	atc	tgt	gac	agc	cga	gtg	ctg	gag	agg	49	
	Ala	Pro	Pro	Arg	Leu	Ile	Cys	Asp	Ser	Arg	Val	Leu	Glu	Arg		
	1				5					10						
tac	ctc	ttg	gag	gcc	aag	gag	gcc	gag	aat	atc	acg	acc	ggc	tgt	gct	97
Tyr	Leu	Leu	Glu	Ala	Lys	Glu	Ala	Glu	Asn	Ile	Thr	Thr	Gly	Cys	Ala	
15					20					25					30	
gaa	cac	tgc	agc	ttg	aat	gag	aac	atc	acc	gtg	cct	gac	acc	aaa	gtg	145
Glu	His	Cys	Ser	Leu	Asn	Glu	Asn	Ile	Thr	Val	Pro	Asp	Thr	Lys	Val	
				35					40					45		
aat	ttc	tat	gcc	tgg	aag	agg	atg	gag	gtt	ggc	cag	cag	gcc	gta	gaa	193
Asn	Phe	Tyr	Ala	Trp	Lys	Arg	Met	Glu	Val	Gly	Gln	Gln	Ala	Val	Glu	
			50					55					60			
gtg	tgg	cag	ggc	ctg	gcc	ctg	ctg	tgc	gaa	gct	gtc	ctg	cgg	ggc	cag	241
Val	Trp	Gln	Gly	Leu	Ala	Leu	Leu	Ser	Glu	Ala	Val	Leu	Arg	Gly	Gln	
		65					70					75				
gcc	ctg	ttg	gtc	aac	tct	tcc	cag	ccg	tgg	gag	ccc	ctg	caa	ctg	cat	289

Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His
80 85 90

gtg gat aaa gcc gtg agt ggc ctt cgc agc ctc acc act ctg ctt cgg 337
Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg
95 100 105 110

gct ctg gga gcc cag aag gaa gcc atc tcc cct cca gat gcg gcc tca 385
Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser
115 120 125

gct gct ccc ctc cgc aca atc act gct gac act ttc cgc aaa ctc ttc 433
Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe
130 135 140

cga gtc tac tcc aat ttc ctc cgg gga aag ctg aag ctg tac aca ggg 481
Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly
145 150 155

gag gcc tgc cgg aca ggg gac aga tgactcgag 514
Glu Ala Cys Arg Thr Gly Asp Arg
160 165

<210> 2
<211> 166
<212> PRT
<213> Homo sapiens

<400> 2
Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu
1 5 10 15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
 145 150 155 160

Cys Arg Thr Gly Asp Arg
 165

<210> 3
 <211> 52
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Oligo1

<400> 3
 ccgggtgccc caccacgcct catctgtgac agccgagtgc tggagaggta cc 52

<210> 4
 <211> 49
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Oligo2

<400> 4
 tcttgaggcc caaggaggcc gagaatatca cgaccggctg tgctgaaca 49

<210> 5
 <211> 52
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Oligo3

<400> 5
 ctgcagcttg aatgagaaca tcaccgtgcc tgacaccaaa gtgaatttct at 52

<210> 6
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Oligo4

<400> 6
 gcctggaaga ggatggaggt tggccagcag gccgtagaag tgtggcag 48

<210> 7

<211> 51
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo5

 <400> 7
 ggccctggccc tgctgtcgga agctgtcctg cggggccagg ccctgttggt c 51

 <210> 8
 <211> 49
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo6

 <400> 8
 aactcttccc agccgtggga gccctgcaa ctgcatgtgg ataaagccg 49

 <210> 9
 <211> 52
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo7

 <400> 9
 tgagtggcct tcgcagcctc accactctgc ttcgggctct gggagcccag aa 52

 <210> 10
 <211> 48
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo8

 <400> 10
 ggaagccatc tcccctccag atgcggcctc agctgctccc ctccgcac 48

 <210> 11
 <211> 53
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo9

 <400> 11
 aatcactgct gacactttcc gcaaactctt ccgagtctac tccaatttcc tcc 53

<210> 12
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo10

<400> 12
ggggaaagct gaagctgtac acaggggagg cctgccggac aggggacaga tgactcgag 59

<210> 13
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo11

<400> 13
tcttggaggc caaggaggcc gagcagatca cgaccggctg tgctgaaca 49

<210> 14
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo12

<400> 14
ctgcagcttg aatgagcaga tcaccgtgcc tgacaccaa gtgaatttct at 52

<210> 15
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo13

<400> 15
cagtcttccc agccgtggga gccctgcaa ctgcatgtgg ataaagccg 49

<210> 16
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligo14

<400> 16
ggaagccatc tccccctccag atgcggccgc agctgctccc ctccgcac

48

<210> 17
<211> 232
<212> PRT
<213> Homo sapiens

<220>
<223> Human IgG1 Fc region-mature protein

<400> 17
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
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Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
130 135 140
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220
Ser Leu Ser Leu Ser Pro Gly Lys

225

230

<210> 18
 <211> 326
 <212> PRT
 <213> Homo sapiens

<220>

<223> Human IgG2 constant region (CH1, hinge, CH2,
 Ch3) - mature protein

<400> 18

Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg
1				5					10					15	
Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr
			20					25					30		
Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser
		35					40					45			
Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser
	50					55					60				
Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Asn	Phe	Gly	Thr	Gln	Thr
65					70					75					80
Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys
			85						90					95	
Thr	Val	Glu	Arg	Lys	Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro
			100					105						110	
Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
		115					120					125			
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
	130					135					140				
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly
145					150					155					160
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn
				165					170					175	
Ser	Thr	Phe	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Val	His	Gln	Asp	Trp
			180					185					190		
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro
		195					200					205			
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Thr	Lys	Gly	Gln	Pro	Arg	Glu
	210					215					220				
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn
225					230					235					240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 245 250 255
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 260 265 270
 Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
 275 280 285
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 290 295 300
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
 305 310 315 320
 Ser Leu Ser Pro Gly Lys
 325

<210> 19
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Linker

<400> 19
 Ala Ala Ala Ala
 1

<210> 20
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Linker

<400> 20
 Ala Ala Ala Ala Ala
 1 5

<210> 21
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Linker

<400> 21
 Gly Gly Gly Gly
 1

<210> 22
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Linker

<400> 22
Gly Gly Gly Gly Gly
1 5

<210> 23
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Linker

<400> 23
Gly Gly Gly Gly Gly Gly Gly
1 5

<210> 24
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Linker

<400> 24
Gly Gly Pro Gly Gly
1 5

<210> 25
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Linker

<400> 25
Gly Gly Gly Gly Ser
1 5

<210> 26
<211> 25
<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Linker

<220>

<223> This linker sequence may encompass five to twenty five amino acids is groups of (GGGGS)

<400> 26

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 27

<211> 53

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Oligonucleotide

<400> 27

aatcactgct gacactttcc gcaaactcct ccgagtctac tccgcattcc tcc 53

<210> 28

<211> 25

<212> PRT

<213> Homo sapiens

<400> 28

Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn
1 5 10 15

Glu Asn Ile Thr Val Pro Asp Thr Lys
20 25

<210> 29

<211> 21

<212> PRT

<213> Homo sapiens

<400> 29

Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Cys Glu Pro Leu Gln
1 5 10 15

Leu His Val Asp Lys
20